#19

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I further certify that the name of the applicant has been amended to WESTERN HEALTH CARE NETWORK pursuant to the provisions of Section 104 of the Patents Act 1990.

WITNESS my hand this
Eighth day of May 2001

CASSANDRA RICHARDS
TEAM LEADER EXAMINATION
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Western Health Care Network.
~~Victorian Infectious Diseases Reference Laboratory~~

AUSTRALIA

Patents Act 1990

PROVISIONAL SPECIFICATION

for the invention entitled:

"Viral Variants and Methods for Detecting Same"

The invention is described in the following statement:

VIRAL VARIANTS AND METHODS FOR DETECTING SAME

The present invention relates generally to viral variants exhibiting reduced sensitivity to particular agents. More particularly, the present invention is directed to hepatitis B variants exhibiting resistance to nucleoside analogues. The present invention further contemplates assays for detecting such viral variants which assays are useful in monitoring anti-viral therapeutic regimums.

Bibliographic details of the publications numerically referred to in this specification are collected at the end of the description. Sequence Identity Numbers (SEQ ID NOs.) for the nucleotide and amino acid sequences referred to in the specification are defined following the bibliography.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

Specific mutations in an amino acid sequence are represented herein as "Xaa_1nXaa_2" where Xaa_1 is the original amino acid residue before mutation, n is the residue number and Xaa_2 is the mutant amino acid.

Hepatitis B Virus (HBV) can cause debilitating disease conditions and can lead to acute liver failure. HBV is a DNA virus which replicates *via* an RNA intermediate and utilizes reverse transcription in its replication strategy (1). The presence of an HBV DNA polymerase has lead to the proposition that nucleoside analogues could act as effective anti-viral agents. Examples of nucleoside analogues currently being tested are penciclovir and its oral form famciclovir (2, 3, 4, 5) and lamivudine (6,7). There is potential for such agents to be used

in the treatment of chronic HBV infection.

Peniciclovir has been recently shown to have potent inhibitory activity against duck HBV DNA synthesis *in vitro* and has been shown to inhibit HBV DNA polymerase-reverse transcriptase activity *in vitro* (8,9). Similarly, oral famiciclovir has been demonstrated to inhibit intra-hepatic replication of duck HBV virus *in vivo* (10). In man, famciclovir has been shown to reduce HBV DNA replication in a patient with severe hepatitis B following orthotopic liver transplantation (OLT) (11).

In work leading up to the present invention, the nucleoside analogue antiviral therapy was used to control severe post-OLT recurrence of HBV infection (12). Long term therapy is mandatory where patients are immunosuppressed and the rate of HBV replication is very high. However, under such conditions, as with any long term chemotherapy of infectious agents, there is a potential for development of resistance or reduced sensitivity to the therapeutic agents employed.

In accordance with the present invention, the inventors have identified variants of HBV with mutations in the HBV DNA polymerase gene which to varying extents reduce the sensitivity of HBV to nucleoside analogues. The identification of these HBV variants is important for the development of assays to monitor nucleoside analogue therapeutic regimums and to screen for agents which can mask the effects of the mutation.

Accordingly, one aspect of the present invention is directed to a variant of an isolated DNA virus which replicates *via* an RNA intermediate wherein said variant comprises a nucleotide mutation in a gene encoding a DNA polymerase or part thereof resulting in at least one amino acid addition, substitution or deletion to said DNA polymerase.

Preferably, the DNA virus is a hepatitis virus and is most preferably HBV.

Preferably, the mutation in the DNA polymerase results in decreased sensitivity of the HBV to a nucleoside analogue.

Regions of the HBV polymerase show amino acid similarity with other RNA-dependent DNA polymerases and RNA-dependent polymerases (13). In this specification, reference is made to the conserved regions defined by Poch *et al* (13) as domains B and C.

Preferably, the mutation results in an altered amino acid sequence in the B domain and/or C domain or regions proximal thereto of the HBV DNA polymerase. The present invention does not extend to a mutation alone in the YMDD motif of the C domain of the HBV DNA polymerase although such a mutation is contemplated by the present invention if it occurs in combination with one or more mutations in another location.

According to a preferred aspect of the present invention, there is provided an HBV variant comprising a mutation in the nucleotide sequence encoding a DNA polymerase resulting in an amino acid addition, substitution and/or deletion in said DNA polymerase in its B domain and/or C domain or in a region proximal thereto, provided said mutation is not in the YMDD motif of the C domain alone, and wherein said variant exhibits decreased sensitivity to a nucleoside analogue.

The nucleoside analogues contemplated by the present invention include penciclovir and its oral form famciclovir as well as lamivudine (3TC). Different variants may be resistant to different nucleoside analogues. For example, in one embodiment, a variant in the B domain of HBV DNA polymerase may be resistant to famciclovir whereas a variant in the C domain may be resistant to 3TC.

The B domain is considered to comprise amino acid residues 488 to 514 of HBV DNA polymerase. This sequence is represented as follows:

I/V L/M GFRKIPMG V/G GLSPFLLAQFTSAICS.

Reference to the B domain includes reference to proximal regions which includes up to about 20 amino acids on either side of the domain.

The C domain comprises amino acids 526 to 536 as follows:

AFSYMDD V/L/M VLG.

This includes the YMDD domain.

Reference to the C domain includes proximal regions of up to 20 amino acids either side of the domain.

The term "resistance" is used in its most general sense and includes total resistance or partial resistance or decreased sensitivity to a nucleoside analogue.

Preferably the variants are in isolated form such that they have undergone at least one purification step away from naturally occurring body fluid. Alternatively, the variants may be maintained in isolated body fluid or may be in DNA form. The present invention also contemplates infectious molecular clones comprising the genome or parts thereof from a variant HBV.

Preferred mutations in the HBV variants include one or more of Ile488Val, Phe491Leu, Val499Leu, Pro503Leu, Leu506Met, Ile512Leu, Met530Val/Ile and/or Ser539Thr. More preferably, the variants contain two or more of the above-mentioned mutations.

According to another aspect of the present invention, there is provided a variant HBV comprising a nucleotide sequence which encodes a DNA polymerase having the amino acid sequence:

$$SHPI_1X_2GX_3RKIPMGX_4GLSX_5$$

$$FLX_6AQFTSAX_7CS \ldots\ldots$$

$$AFSYX_8DDX_9VLGAKX_{10}$$

wherein X_1 is I or V;
X_2 is L or M;
X_3 is F or L;
X_4 is L or V or G;
X_5 is P or L;
X_6 is M or L;
X_7 is I or L;
X_8 is M or I or V;
X_9 is V or L or M; and/or
X_7 is S or T;

and wherein said variant exhibits reduced sensitivity to a nucleoside sensitivity to a nucleoside analogue, such as famciclovir (penciclovir) and/or lamivudine (3TC).

Examples of preferred variants comprise the amino acid sequences shown in Figure 3.

The identification of the variants of the present invention permits the generation of a range of assays to detect such variants. The detection of such variants may be important in identifying resistant variants to determine the appropriate form of chemotherapy.

Accordingly, another aspect of the present invention contemplates a method for determining the potential for an HBV to exhibit reduced sensitivity to a nucleoside analogue, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding HBV DNA polymerase resulting in at least one amino acid substitution, deletion and/or addition in the B domain or C domain or a region proximal thereto of said DNA polymerase wherein the presence of such a mutation is an indication of the likelihood of resistance to said nucleoside analogue.

Preferably, the assay determines a mutation resulting in a Glu/Val499Leu substitution and/or a Leu506Met substitution and/or a Pro503Leu and/or a S539T, respectively.

The DNA or corresponding RNA may be assayed or alternatively, the DNA polymerase itself may be screened for the mutation.

The detection according to this aspect of the invention may be any nucleic acid-based detection means, for example nucleic acid hybridisation techniques or polymerase chain reaction (PCR). The invention further encompasses the use of different assay formats of said nucleic acid-based detection means, including restriction fragment length polymorphism (RFLP), amplified fragment length polymorhism (AFLP), single-strand chain polymorphism (SSCP), amplification and mismatch detection (AMD), interspersed repetitive sequence polymerase chain reaction (IRS-PCR), inverse polymerase chain reaction (iPCR) and reverse transcription polymerase chain reaction (RT-PCR), amongst others.

The present invention extends to a range of immunologically based assays to detect variant HBV DNA polymerase. These assays are based on antibodies directed to naturally occurring HBV DNA polymerase which do not, or substantially do not, interact with the variant HBV DNA polymerase. Alternatively, antibodies to a variant HBV DNA polymerase are used which do not or substantially do not, interact with naturally occurring HBV DNA polymerase.

Monoclonal or polyclonal antibodies may be used although monoclonal antibodies are preferred as they can be produced in large quantity and in a homogenous form. A wide range of immunoassay techniques are available such as described in U.S. Patent Nos. 4,016,043, 4,424,279 and 4,018,653.

The present invention further contemplates agents which mask the nucleoside analogue resistance mutation. Such agents will be particularly useful in long term treatment by nucleoside analogues. The agents may be DNA or RNA or proteinaceous or non-proteinaceous chemical molecules. Natural product screening such as from plants, coral and microorganism is also contemplated as a useful potential source of masking agents. The agents may be in isolated form or in the form of a pharmaceutical composition and may be administered sequentially or simultaneously with the nucleoside analogue.

The subject invention extends to kits for assays for variant HBV. Such kits may, for example, contain the reagents from PCR or other nucleic acid hybridisation technology or reagents for immunologically based detection techniques.

The present invention is further described by the following non-limiting figures and examples.

In the figures:

Figure 1 is a graphic representation showing serum biochemical (ALT) and virological (HBV DNA) profile in the transplant patient and the responses following the introduction of various antiviral treatment programs. Treatment GCV + PFF, GCV and FCV[I] and FCV[II] are described in detail in the examples. Treatment GCV + PFF is ganciclovir plus foscarnet combination (12), treatment GCV is parenteral ganciclovir maintenance therapy and treatment FCV[I] and FCV[II] is oral famciclovir therapy at a dose of 250 mg or 500 mg twice daily, respectively. The day each therapy commenced is shown in brackets. The ALT (•-•) and the HBV DNA (□-□) responses are plotted against time from the commencement of antiviral therapy at 6 months post-OLT. The five key time points for the sequence analysis, pre-treatment (PRE-) and days 87, 600, 816 and 1329 post antiviral treatment are shown.

Figure 2 is a representation showing amino acid alignment of the RNA dependent DNA polymerase sequence motifs from HBV, pre-treatment with famciclovir and 370 days post-treatment (total antiviral therapy of 816 days), with the woodchuck hepatitis virus (WHV), human immunodeficiency virus (HIV), and the comparable regions with the DNA polymerase of herpes simplex virus (HSV) (13, 14). The conserved asparagine (D) and glycine (G) residues within the polymerase motifs are in bold type and the amino acid changes found after famciclovir treatment are in bold type and underlined. The location of the domains and the mutated amino acid residues within HBV polymerase are shown. The bold face underlined glycine (G) residue in the HSV polymerase becomes a cysteine (C) during penciclovir treatment (15).

Figure 3 is a representation showing amino acid alignment of the RNA dependent DNA polymerase sequence motifs from HBV, noting the amino acid changes which have been selected for in the presence of famciclovir and 3TC. HBV concensus sequence was derived from published sequences in Genebank/Entrez. The conserved asparagine (D) and glycine (G) residues within the polymerase motifs are in bold type. The amino acid changes found after famciclovir treatment are in bold green type and underlined and after 3TC are in bold blue type and are underlined. The amino acid sequence of the HBV isolated from patient A (HBV post treatment sequence from Figure 2) and patient B, during famicilovir treatment and from Patient C who did not respond to famciclovir and was later treated with 3TC in which a resistance mutation was selected (3TC 2). The published 3TC changes detected by Ling *et al* (16) is shown in 3TC 1.

EXAMPLE 1

CASE STUDY

1. PATIENT A

The inventors sequenced the HBV polymerase and X open reading frames from a series of isolates from a patient who received antiviral therapy for almost 4 years following post liver transplant recurrence of HBV infection (Figure 1).

The patient (male, aged 42 years) was transplanted because of end-stage liver failure due to chronic HBV infection. The initial post transplant course was unremarkable but by 5 months there was evidence of recurrent infection and very high levels of viral replication and deteriorating liver function (12). The histological picture was consistent with fibrosing cholestatic hepatitis. Antiviral treatment was commenced approximately 6 months post-OLT. Initially, the patient received intravenous (iv) ganciclovir (GCV; 10 mg/kg/day) in combination with iv foscarnet (PFF; 50-125 mg/kg/day; the dose depending on renal function) (12). This is the treatment of GCV+ PFF described in Figure 1 which lasted for 86 days. Maintenance iv GCV (3.3-6.7 mg/kg/day) three times per week was commenced on day 87 of antiviral treatment (GCV in Figure 1). Oral famciclovir (250 mg, twice daily)

was commenced on day 446 of therapy (FCV [I] in Figure 1) which was increased to 500 mg twice daily (FCV [II] in Figure 1) on day 500. The patient is currently on this treatment regime. The clinical and virological details of this patient preceding famciclovir therapy have been reported (12).

Serum samples were routinely collected and stored at -70°C. Informed consent was obtained from the patient to use these samples for research purposes. Figure 1 shows the alanine amino transferase (ALT) and HBV DNA levels over the entire course of antiviral treatment. The 5 samples chosen for additional studies cover a period of almost four years.

2. PATIENT B

Patient B was retransplanted for pre-core mutant associated HBV-related allograph loss 14 months after the initial liver transplant. Antiviral treatment with GCV (7.5 mg/kg/day) was given for 10 months and then ceased. This was followed by oral famciclovir therapy given (500 mg 3 times/day).

From patient B the entire HBV polymerase gene was sequenced from a serum HBV sample taken post-transplantation after 850 days FCV therapy. The regions encompassing the catalytic domains of the HBV polymerase were sequenced from a serum sample pretransplant prior to FCV treatment.

3. PATIENT C

This patient did not respond to famciclovir and was later treated with lamivudine (3TC) (6, 7) in which a resistance mutation was selected.

EXAMPLE 2
VIRAL MARKERS IN SERUM

Hepatitis B surface antigen (HbsAg), hepatitis B e antigen (HbeAg), anti-HBe, hepatitis B core antigen (HbcAg) specific IgG and IgM, hepatitis A specific IgM, hepatitis delta antigen and antibody, and anti-hepatitis C virus antibody were measured using commercially available immunoassays (Abbott Laboratories, North Chicago, IL). Only the HBV markers were positive. Hepatitis B viral DNA levels were measured and quantified using a capture hybridisation assay according to the manufacturer's directions (Digene Diagnostics Inc., Beltsville, MD). This patient was infected with a pre-core HBV mutant pre-OLT (12) and this status did not change post-OLT.

EXAMPLE 3
SEQUENCING AND CLONING OF HBV DNA

1. **Extraction of DNA from sera:** Aliquots of 50 μl of sera were mixed with 150 μl TE (10 mmol/L Tris-HCl (pH 7.5), 2 mmol/L EDTA), 1% w/v sodium dodecyl sulfate and 1 mg/ml pronase and incubated at 37°C for 2 hours. DNA was deproteinised by phenol/chloroform, precipitated with isopropanol and dissolved in 25 μl nuclease-free water.

2. **Amplification of the viral polymerase and X genes by polymerase chain reaction (PCR):** Oligonucleotides were synthesised by Bresatec, Adelaide, Australia. For amplification of the polymerase gene, the sense primer was 5'- GGA GTG TGG ATT CGC ACT CC -3' [SEQ ID NO:1] (nucleotides [nt] -40 to -21) and the antisense primer was 5'- GCT CCA AAT TCT TTA TA -3' [SEQ ID NO:2] (nt 2831 to 2847). For amplification of the X gene, the sense primer was 5'-CCT TTA CCC CGT TGC CCG GC -3' [SEQ ID NO:3] (nt 2055 to 2074) and the antisense primer 5'- GCT CCA AAT TCT TTA TA -3' [SEQ ID NO:4] (nt 2831 to 2847). All nt are numbered from the start of the polymerase gene. Each reaction was carried out using 5 μl of the extracted DNA as template, 1.5 U of *Taq* polymerase (Perkin Elmer Cetus, Norwalk, CT), 1 μmol/L of sense and antisense

primers, 200 μmol/L each of deoxynucleoside triphosphates, 50 mmol/L Kcl, 3.5 mmol/L MgCl, 10 mmol/L Tris-Hcl (pH 8.3) and 0.01% w/v gelatin. Amplification was achieved by 40 cycles of denaturation (94°C for 1 min), annealing (55°C for 1 min) and extension (72°C for 1.5 min), followed by a final extension of 7 min (Perkin-Elmer Cetus, Norwalk, CT). The PCR product was analysed by gel electrophoresis through 1.5 % w/v agarose and visualised by UV irradiation after staining with ethidium bromide.

3. Sequencing of the polymerase and X genes of HBV DNA: The specific amplified product was purified using Geneclean II (BIO 101 Inc., La Jolla, CA) and directly sequenced using Sequenase version 2.0 (United States Biochemical Corp., Cleveland, OH). The PCR primers were used as sequencing primers and internal primers were additionally synthesised to sequence the internal regions of the PCR products. The following internal and sequencing primers were used 5′- GCC GCG TCG CAG AAG ATC TCA AT -3′ [SEQ ID NO:5] (nt 104-126), 5′- GGT TCT ATC CTA ACC TTA CC -3′ [SEQ ID NO:6] (nt 341-360), 5′- GCC TCA TTT TGT GGG TCA CCA TA -3′ [SEQ ID NO:7] (nt 496-518), 5′- TGG GGG TGG AGC CCT CAG GCT -3′ [SEQ ID NO:8] (nt 731-751), 5′- CAC AAC ATT CCA CCA AGC TC -3′ [SEQ ID NO:9] (nt 879-899), 5′- AAA TTC GCA GTC CCC AAC -3′ [SEQ ID NO:10] (nt 1183-1195), 5′- GTT TCC CTC TTC TTG CTG T -3′ [SEQ ID NO:11] (nt 1429-1447), 5′- TTT TCT TTT GTC TTT GGG TAT -3′ [SEQ ID NO:12] (nt 1683-1703) 5′-CCA ACT TAC AAG GCC TTT CTG-3′ [SEQ ID NO:13] (nt 1978-1999), 5′-CAT CGT TTC CAT GGC TGC TAG GC-3′ [SEQ ID NO:14] (nt 2239-2262).

4. Cloning of the HBV polymerase gene into pUC18:

Due to the small amount of HBV DNA in the samples, the region encompassing nt 1429 to 1703 from the HBV polymerase gene were amplified by PCR using the primers -5′-GTT TCC CTC TTC TTG CTG T-3′ [SEQ ID NO:15] (nt 1429-1447) and 5′ ATA CCC AAA GAC AAA AGA AAA- 3′ [SEQ ID NO:16] (nt 1703-1683), before cloning. The DNA was purified with Geneclean II and ligated using T4 DNA ligase (New England Biolabs, Beverly, MA) into a *Sma* I - digested dephosphorylated pUC18 plasmid (Pharmacia Biotech, NJ). Clones were directly sequence as above.

EXAMPLE 4

DNA POLYMERASE ASSAY

Samples of serum (100 μl) were applied to a 20% w/v sucrose cushion in TNE (20 mmol/L Tris-HCl pH 7.4, 150 mmol/L $NaCl_2$ 1 mmol/L EDTA) and centrifuged at 200,000 g for 3 hr at 10°C using an SW41 rotor in a Beckman Model L8 ultracentrifuge. The pellet was resuspended in 50 mmol/L Tris-HCl pH 7.5 containing 1.5% v/v Triton-X100, 100 mmol/L Kcl and 0.01% v/v 2-mercaptoethanol and allowed to stand overnight at 4°C. Small aliquots of the suspension were assayed for endogenous HBV DNA polymerase activity essentially as described by Price *et al* (16). Each assay was performed in a total volume of 30 μl which contained 20 μl of the partly purified HBV and (final concentrations) 30 mmol/L Tris-HCl pH 7.5, 30 mmol/L $MgCl_2$, 10 μmol/L each dATP, dTTP and dGTP, and 0.01 μM [α-^{32}P]-dCTP (3,000 Ci/mmol) (Dupont NEN, Boston, MA). To test for penciclovir triphosphate (PCV-TP) sensitivity, paired assays were performed on each sample, with an excess (100 μmol/L penciclovir-triphosphate included in the reaction mixture in one assay of each pair. After 2 hr at 37°C, reactions were stopped by spotting 20 μl aliquots of each reaction mix onto 25mm diameter glass fibre discs (Advantex, Tokyo, Japan) which had been pre-soaked in 10% w/v trichloroacetic acid (TCA). Discs were dried before washing in ice-cold 10% w/v TCA containing 10 mmol/L sodium pyrophosphate. Three further 10 min washes in cold 5% v/v TCA followed. The washed discs were finally rinsed in absolute ethanol, air dried, and counted for radioactivity. Inhibition of HBV DNA polymerase activity by PCV-TP was expressed as the percentage difference in activity in the assay mix containing PCV-TP compared to the matched control. Because of limited sample amounts, it was not possible to standardize enzyme activity or to perform replicate assays. Despite the inherent variability of the assay, a general time related decrease in sensitivity of the HBV DNA polymerase to PCV-TP was evident (see Table 1).

EXAMPLE 5

EFFECT OF ANTIVIRAL THERAPY

Upon commencement of the antiviral treatment strategy GCV+ PFF, the level of HBV DNA post-OLT decreased from over 100,000 pg/ml to 10,800 pg/ml by day 87 (Figure 1). This reduction in viraemia was associated with clinical, biochemical and histological improvement (12). Maintenance famciclovir therapy (treatment GCV) resulted in fluctuating levels of HBV DNA over the ensuring 359 days with two peaks of HBV DNA observed. The switch to oral famciclovir on day 446 was also associated with a rise in HBV DNA, but this was likely to have been the result of insufficient dosing (FCV[I] in Figure 1) rather than a breakthrough in treatment. Following dose increase to FCV [II] on day 500, there was a decrease in HBV DNA. However, the level of HBV DNA gradually rose over time from 3,000 pg/ml on day 600 (154 days of famciclovir), to 8,800 pg/ml on day 816 (370 days famciclovir), peaking at 29,000 pg/ml on day 1302 (856 days of famciclovir), then stabilising at around 12,000 pg/ml on day 1329 (883 days of famciclovir). A students test of the DNA levels during the treatment period from days 816 to days 1329, revealed statistically significant rise. There was a 1.5 to 2 fold rise in ALT levels over the same time interval (Figure 1) and no change in clinical status.

EXAMPLE 6

NUCLEOTIDE CHANGES

The X and the polymerase genes of HBV were sequenced at five time points (Figure 1). During almost 4 years of the antiviral therapy there were no changes in the X gene compared to the pretreatment sequence. However, there were 5 nt changes detected in the polymerase gene from day 816 and day 1329 samples (Table 1). These changes were detected in separate independent PCR amplifications; furthermore the mutations were detected by sequencing both strands and are therefore unlikely to be the result of PCR generated errors. The nt changes in the polymerase gene were first detected after 816 days of treatment, when the patient had been treated with famciclovir for 370 days. However, only two nt changes, at positions 1498

and 1519 resulted in amino acid changes, Val 499- Leu and Leu 506- Met, respectively. These two nt changes appeared concurrently. At 816 days, three different nt (C,G,T) were detected at position 1498 (all of which would result in a Val to Leu change). After 1329 days post-treatment, thymidine was the dominant species at nt 1498. The amino acid changes at 816 and 1329 days post treatment coincided with reduced serum HBV DNA polymerase sensitivity to PCV-TP (Table 1). These nt changes were not found in 6 patients with post-OLT recurrent HBV infection who were not undergoing FCV therapy.

The region encompassing the nt mutations which gave rise to amino acid changes from the sample taken at 1329 days was cloned and sequenced. Three quasi-species were detected. Seventy-five percent (15/20) of the clones contained both the 1498 and 1519 mutations which occurred together. Pretreatment non-mutated sequences were detected in 3/20 of the clones. A further mutation at nt 1511, which would result in a proline to leucine change at position 503, was detected in 2/20 of the clones. This mutation was not detected with the two dominant mutations, 1498 (Val 499-Leu) and 1519 (Leu 506-Met), nor was it detected by direct PCR sequencing, indicating it probably occurs at a low frequency. Viral DNA from the sample obtained at 600 days (150 days of FCV treatment) was also cloned and sequenced; however, only the pre-treatment sequences were detected.

EXAMPLE 7

NUCLEOTIDE CHANGES IN PATIENTS B AND C

The amino and changes in HBV isolated from patient B and C are shown in Figure 3.

Patient B was undergoing long term famciclovir treatment (>850 days).

Patient C did not respond to famciclovir and was later treated with 3TC (lamivudine [6,7]). In Figure 3, famciclovir 1 is from Figure 2 and famciclovir 2 is from Patient B. 3TC2 is the resistance mutation sequence from Patient B. The sequence analysis showed a mutation in the HBV polymerase gene was near the C domain but not in the YMDD motif. The specific

match is a Thr→Ser substitution.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

TABLE 1

Nucleotide mutations in the polymerase gene and the resulting amino acid changes during antiviral therapy

Days of antiviral treatment	Days post famciclovir treatment	POLYMERASE GENE nt 297	nt 1498	nt1511*	nt 1519	nt 2008	nt 2331	Inhibition of HBV DNA Polymerase by PCV-TP**
Pretreatment	NR***	T	G	C	C	C	G	40 %
87	NR	-	-	-	-	-	-	NA****
600	154	-	-	-	-	-	-	30 %
816	370	-	G, T , C	-	A	-	-	0 %
1329	883	C	T	T	A	A	A	0 %
Amino acid change		None	Val 499-Leu	Pro 503-Leu	Leu 506-Met	None	None	

The dashes indicate no change from the pre-treatment nucleotide.

* The mutation was only detected after cloning the PCR product after 1329 days of antiviral treatment. It occurred at a low frequency and was present in only 10% of clones.

** The percentage inhibition of HBV DNA polymerase by PCV-TP in the *in vitro* assay as described in the Methods section.

*** NR- not relevant

**** NA- not assessable

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: VICTORIAN INFECTIOUS DISEASES REFERENCE LABORATORY

(ii) TITLE OF INVENTION: VIRAL VARIANTS AND METHODS FOR DETECTING SAME

(iii) NUMBER OF SEQUENCES: 16

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: AU PROVISIONAL
(B) FILING DATE: 08-NOV-1996

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGA GTG TGG ATT CGC ACT CC 20

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GCT CCA AAT TCT TTA TA 17

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CCT TTA CCC CGT TGC CCG GC 20

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GCT CCA AAT TCT TTA TA 17

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GCC GCG TCG CAG AAG ATC TCA AT 23

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GGT TCT ATC CTA ACC TTA CC 20

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GCC TCA TTT TGT GGG TCA CCA TA 23

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TGG GGG TGG AGC CCT CAG GCT 21

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CAC AAC ATT CCA CCA AGC TC 20

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

AAA TTC GCA GTC CCC AAC 18

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GTT TCC CTC TTC TTG CTG T 19

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

TTT TCT TTT GTC TTT GGG TAT 21

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CCA ACT TAC AAG GCC TTT CTG 21

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CAT CGT TTC CAT GGC TGC TAG GC 23

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GTT TCC CTC TTC TTG CTG T 19

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

ATA CCC AAA GAC AAA AGA AAA 21

DATED this 8th day of November, 1996

Western Health Care Network.

~~VICTORIAN INFECTIOUS DISEASES REFERENCE LABORATORY~~

By Its Patent Attorneys

DAVIES COLLISON CAVE

FIGURE 1

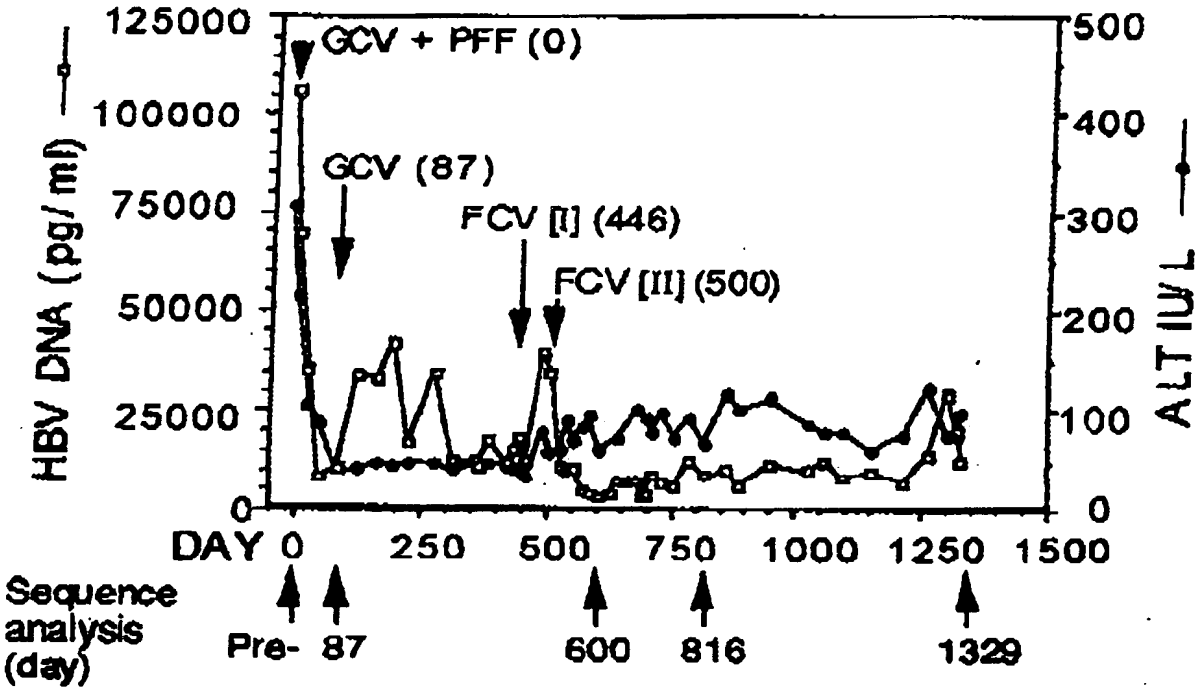

125000
100000
75000
50000
25000
0
HBV DNA (pg/ml)
500
400
300
200
100
0
ALT IU/L
GCV + PFF (0)
GCV (87)
FCV [I] (446)
FCV [II] (500)
DAY 0
250
500
750
1000
1250
1500
Sequence analysis (day)
Pre-
87
600
816
1329

FIGURE 2

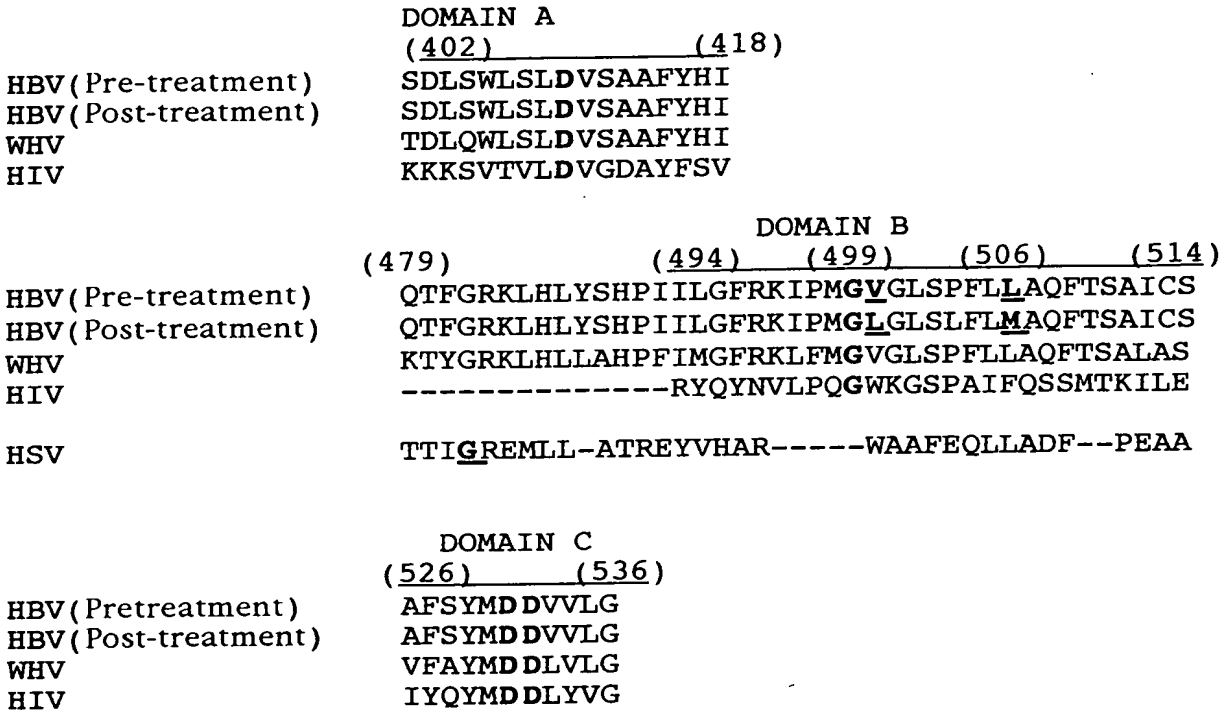
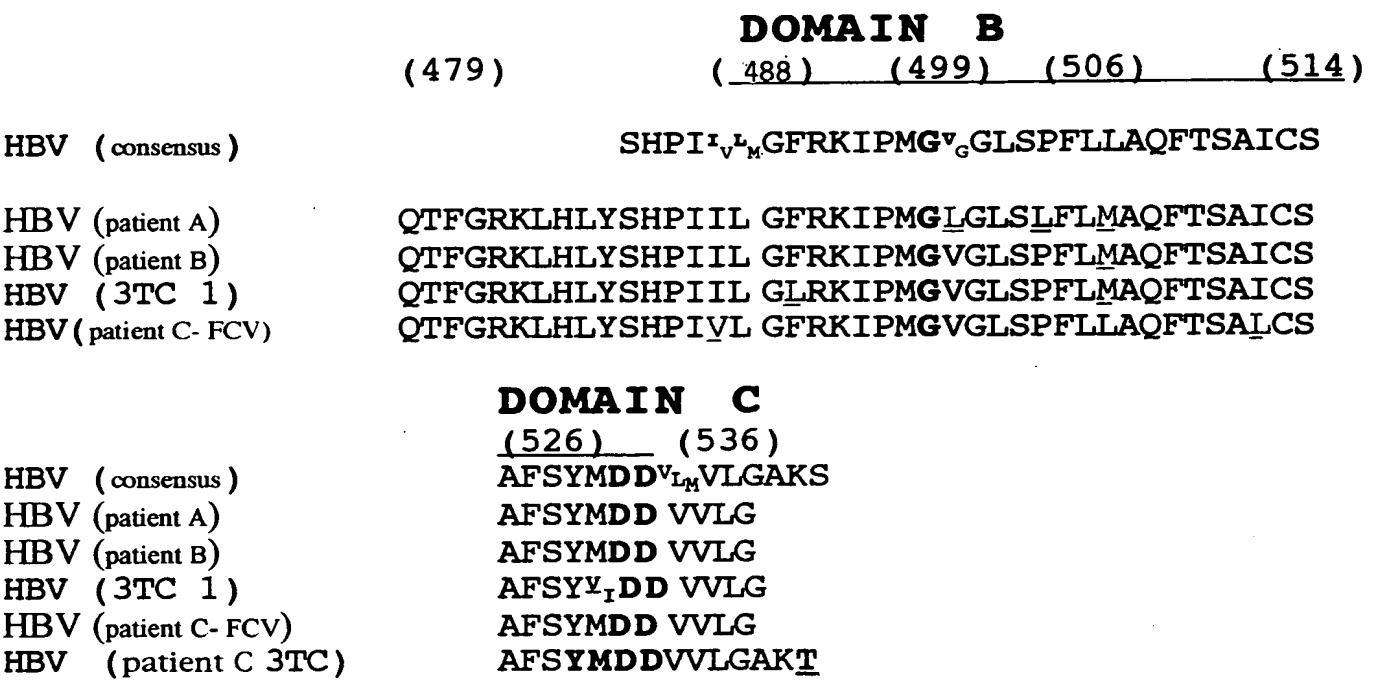

DOMAIN A
(402) (418)
HBV(Pre-treatment) SDLSWLSLDVSAAFYHI
HBV(Post-treatment) SDLSWLSLDVSAAFYHI
WHV TDLQWLSLDVSAAFYHI
HIV KKKSVTVLDVGDAYFSV
DOMAIN B
(479) (494) (499) (506) (514)
HBV(Pre-treatment) QTFGRKLHLYSHPIILGFRKIPMGVGLSPFLLAQFTSAICS
HBV(Post-treatment) QTFGRKLHLYSHPIILGFRKIPMGLGLSLFLMAQFTSAICS
WHV KTYGRKLHLLAHPFIMGFRKLFMGVGLSPFLLAQFTSALAS
HIV ----------RYQYNVLPQGWKGSPAIFQSSMTKILE
HSV TTIGREMLL-ATREYVHAR-----WAAFEQLLADF--PEAA
DOMAIN C
(526) (536)
HBV(Pretreatment) AFSYMDDVVLG
HBV(Post-treatment) AFSYMDDVVLG
WHV VFAYMDDLVLG
HIV IYQYMDDLYVG

FIGURE 3


DOMAIN B
(479)
(488) (499) (506) (514)
HBV (consensus) SHPI[I,V][L,M]GFRKIPMG[V,G]GLSPFLLAQFTSAICS
HBV (patient A) QTFGRKLHLYSHPIIL GFRKIPMGLGLSLFLMAQFTSAICS
HBV (patient B) QTFGRKLHLYSHPIIL GFRKIPMGVGLSPFLMAQFTSAICS
HBV (3TC 1) QTFGRKLHLYSHPIIL GLRKIPMGVGLSPFLMAQFTSAICS
HBV (patient C- FCV) QTFGRKLHLYSHPIVL GFRKIPMGVGLSPFLLAQFTSALCS
DOMAIN C
(526) (536)
HBV (consensus) AFSYMDD[V,I][L,M]VLGAKS
HBV (patient A) AFSYMDD VVLG
HBV (patient B) AFSYMDD VVLG
HBV (3TC 1) AFSY[V,I]DD VVLG
HBV (patient C- FCV) AFSYMDD VVLG
HBV (patient C 3TC) AFSYMDDVVLGAKT